Lymphopenia

ACTH and steroid therapy, Cushing syndrome, Aplastic anemia

Monocytes : 2 -8 %

Monocytosis

Malaria, Kala azar, infectious mononucleosis, monocytic leukemia, tuberculosis, typhoid, typhus, rocky mountain spotted fever, syphilis, some chronic inflammatory conditions.

Monocytopenia : uncommon. Occurs in aplastic anemia

Leukemia

Uncontrolled, abnormal cancerous proliferation of precursors belonging to WBC series resulting in appearance of large number of immature forms of WBC in peripheral circulation. Total WBC count is enormously increased. Depending on the type of WBC series affected, it can be myeloid, lymphatic or monocytic leukemia. Can be acute or chronic.

Agranulocytosis : is the sever absolute decrease in circulating granulocytes below normal range. May be caused by chloramphenicol, phenylbutazone, etc.

Study Arneth count and Schilling index (Schilling's test is different from Schilling index and is used to assay Vitamin B12).

Result

The differential leucocyte count in my own sample is

Neutrophil - 80%
Lymphocyte - 16%
Monocyte - 4%
Basophil - 0%
Eosinophil - 0%

Questions

1. What is a macrophage?
2. Distribution of WBC s in a peripheral smear.
3. What is the importance of doing DLC?
4. What is Cooke - Arneth count and what is importance?
5. Draw different leukocytes

(1) A type of WBC that surrounds & kills microbes and remove dead cells & stimulates the action of other immune system

(2)

Neutrophil - 50-70%
Eosinophil - 1-4%
Lymphocyte - 20-40%
Monocyte - 2-8%
Basophil - 0-1%

(3) Differential leukocyte count test measures the percentage of different WBC types in blood. This test tells about existence of immature WBC in blood that may lead to abnormalities like anemia, leukemia and infection.

(4) The Arneth count determines the percentage of neutrophils with one, two, three, 4 or 5 → more lobes.

redraw

Fine lilac granules

Coarse, bluish black granules

large lymphocyte should have almost same size as neutrophil

N	V	N	L	N	L	N	N	L	N
L	N	L	N	L	N	N	N	L	N
N	L	N	N	L	N	N	N	N	N
N	N	N	N	N	N	L	N	N	N
N	N	L	N	N	N	M	N	N	N
N	N	N	M		N	M	N	N	N
L	N	L	N	N	N	N	N	N	N
L	N	N	L	N	N	N	M	N	N
L	N	L	N	N	N	N	N	N	N
L	L	N		N	N	N	N	N	

Result - DLC result is
Neutrophil - 80%
Lymphocyte - 1[illegible]%
Monocyte - 4%
Basophil 0
Eosinophil - 0

Date :

Experiment 12

BLEEDING TIME

AIM

To determine the bleeding time of a subject

REQUIREMENTS

Lancet, cotton, rectified spirit, filter paper, stop watch

PROCEDURE

Duke's method : Sterilize the fingertip using rectified spirit and allow to dry. Make a sufficiently deep prick using a sterile lancet, so that blood comes out freely without squeezing. Note the time (start the stop-watch) when bleeding starts. Mop the blood by touching fingertip with a filter paper. This is repeated every 15 seconds, each time using a fresh portion of the filter paper, till bleeding stops. Note the time (stop the stop watch). It is seen that the bloodstain on the filter paper get smaller and smaller to disappear finally when bleeding stops.

DISCUSSION

Bleeding time is the interval between the moment when bleeding starts and the moment when bleeding stops. Normal bleeding time (Duke's method) is 1 to 4 minutes. Bleeding time is prolonged in purpuras, but normal in coagulation disorders like hemophilia. Purpuras can be due to

1. Platelet defects –

   Thrombocytopenic purpura – Primary (Idiopathic) and secondary.

   Thrombasthenic purpura.
2. Vascular defects – Senila purpura, henoch Schonlein purpura, scurvy
3. Von Willebrand's disease

   Platelets are important in preventing small vessel bleeding, by causing vasoconstriction and platelet plug formation

Other method

Ivy's method – Apply the sphygmomanometer cuff on the upper arm Raise the cuff pressure and maintain at 40mm of Hg. Under sterile conditions make a deep prick on the foream just below the elbow. Bleeding time is noted as in Duke's method.

Normal value is 2 to 7 minutes

Result

Bleeding time of my own blood is

Questions

1. Other methods in determining bleeding time.
2. What is Purpura?
3. How does bleeding stop from a skin-prick?
4. Conditions in which BT is prolonged?

(1) Ivy's method

(2) Purpura is the spontaneous hemorrhage into the skin and mucous membrane from capillaries due to abnormalities in blood vessels or platelet.

(3) Bleeding stops from a skin prick due to temporary platelet plug formation.

(4) Bleeding time is prolonged in purpura.
Purpura can be due to

(i) Platelet defect → Thrombocytopenia purpura – primary.
Thrombasthenic purpura – secondary.

(ii) Von willebrand's disease

Date :

Experiment 13

CLOTTING TIME

AIM

To determine the clotting time of a subject.

REQUIREMENTS

Fine capillary glass tubes of about 10cm length, cotton, rectified spirit, lancet and stopwatch.

PROCEDURE

Capillary tube method (Wright's method)

Under sterile precautions make a sufficiently deep prick in the finger tip. Note the time when bleeding stats (start the stop watch). Touch the blood drop at the fingertip using one end of the capillary tube kept tilted downwards. The tube gets easily filled by capillary action. After about two minutes start snapping off small lengths of the tube, at intervals of 30 seconds, each time noting whether a fibrin thread is formed between the snapped ends. Note the time (stop the stopwatch, when the fibrin thread is first seen).

DISCUSSION

Clotting time is the interval between the moment when bleeding starts and the moment when the fibrin threads is first seen.

Normal value is 3 to 10 minutes.

Bleeding time and clotting time are not the same. Bleeding time depends on the integrity of platelets and vessel walls. Whereas clotting time depends on the availability of coagulation factors. In coagulation disorders like hemophilia, clotting time is prolonged but bleeding time remains normal. Clotting time is prolonged in conditions like vitamin K deficiency (hemorrhagic disease of newborn, obstructive jaundice), liver diseases, disseminated intravascular coagulation, over dosage of anticoagulants, hypoprothrombinemia, afibrinogenemia, viper envenomation etc. Bleeding time as well as clotting time are prolonged in severe cases of von Willebrand's disease.

Other method: Modified Lee and White method

Under aseptic precaution venepuncture is done and one ml, each of blood is collected in 3 small test tubes. Note the time when blood is taken. Keep the test tubes in a water bath maintained at 37°C. Tilt the tubes every 30 seconds and see whether the blood is flowing. Repeat this till the tube can be inverted without the blood flowing out. Note the time. Average value of the results in the 3 test tubes gives the clotting time.

Normal value is 9 – 11 minutes.

Result

Clotting time of my own blood is 3 minutes

Questions

1. List out the clotting factors.
2. Hemophilia and its types.
3. Physiological anticoagulants.
4. Conditions where CT is increased
5. What is meant by terms thrombosis & embolism?

① Clotting factors are
- Fibrinogen
- Prothrombin
- Thrombokinase
- Ionized calcium
- Stable factor, etc.

incomplete

Clotting factors involved in the intrinsic pathway ~~increase~~ include factors XII, IX, ~~a~~XI, and VIII. Clotting factors involved in ~~th~~ extrinsic pathway include factors VII and III. The common pathway include clotting factors ~~XII~~ X, VI, II, I, and VIII.

② Hemophilia is usually an inherited bleeding disorder in which blood dont clot properly. This can lead to spontaneous bleeding as well as bleeding following injuries/surgery.

There are also other types of hemophilia, the most common one are

Hemophilia A: (classic hemophilia) :- this type is caused by a lack ~~of~~ or decrease in clotting factor VIII

Hemophilia B: (Christmas disease) this type is caused by a lack or decrease in clotting factor IX

③ Anticoagulant types include Heparin, which is
- injected, ~~a~~ which activates antithrombin III and inhibits thrombin and clotting factor X.
- Coumarin derivatives are oral anticoagulants

that binds to and inhibits the activity of thrombin ~~and factor Xa~~. hence dissolving blood clots.

4) Circumstance risk factor including central venous) ~~warfarins~~ catheters, trance, combined oral contraceptive. oral HRT, leg injury and advancing age. CT ↑ ?

5) Thrombosis occurs when blood clots block veins or arteries symptoms include pain and swelling in one leg, chest pain/et.

Air embolism is a blocked artery caused by a foreign body such as a blood clot/ an air bubble. The body's tissue and organs need O_2 which is transported around the body to the bloodstream.

Experiment 14

Date :

BLOOD GROUPING

AIM

To determine the blood group of a person – A B O system and Rh type.

PRINCIPLE

The blood group is determined by observing the reaction between the red cells and a sample of serum containing a known agglutinin.

REQUIREMENTS

Glass slides, Anti A, Anti B, Anti D sera, citrated saline, rectified spirit watch glass, lancet.

Anti A serum is blue in colour

Anti B serum is yellow in colour

Anti D serum is colourless

PROCEDURE

Take 1 ml of citrated (1% sodium citrate solution in 0.9% saline) saline in a clean and dry watch glass. Citrate acts as anticoagulant. Under sterile precautions, prick the finger tip and add 3 drops of blood to the citrated saline. Mix well by gently rocking the watch glass. Thus a uniform red cell suspension is obtained. Take 4 glass slides and mark Anti A, Anti B, Anti D and C against the slides. Put one drop each of Anti A serum, Anti B Serum, Anti D serum and normal saline in the corresponding slides. Add one drop of red cell suspension to each of the four slides, without the dropper touching the sera. Mix the red cell suspension and serum. Wait for 8 to 10 minutes. At the end of 10 minutes, mix gently and look for any agglutination.

OBSERVATION

If agglutination is present, red cells are seen clumped together (sprinkled chilli powder appearance). This is usually visible with the naked eye. In doubtful cases, the result may be confirmed by microscopic examination.

If there is agglutination in slide A, the blood group is A, if there is agglutination in slide B, the blood group is B. If there is agglutination in both A and B, the group is AB and if there is no agglutination in A or B, the group is O.

If there is agglutination in slide D, the Rh type is positive and if there is no agglutination it is Rh negative Normal saline is used a s control to check any agglutination due to contamination or auto agglutination.

DISCUSSION

In the A B O system individuals are classified into A, B, AB and O groups depending on the presence or absence of agglutinogens A and B in the red cells.

Blood Group	Agglutination	Agglutinin
A	A	Anti B
B	B	Anti A
AB	A, B	-
O	-	Anti A, Anti B

In Rh system individuals are classified into Rh positive and Rh negative depending on the presence or absence of Rh antigen (D antigen).

Significance of blood grouping: Blood transfusion, Medico-legal importance, Disputed paternity, Proneness to certain diseases, erythroblastosis fetalis, anthropological studies.

PRECAUTIONS

1. Separate droppers should be used for the different sera
2. Agglutination should be observed before the mixture gets dried up.
3. If agglutination occurs in control, it may be due to contamination or autoagglutination.

Clean the slide and watch glass; and repeat the procedure. If agglutination still occurs it is due to autoagglutination by antibodies present in subject's blood. C is the control for comparison. In doubtful cases of –ve test, use hand lens. If still doubt, take a drop from the glass slide; put a coverslip; and examine under microscope. In polycythemia, rouleaux may give false impression of agglutination. Rouleaux and agglutination can be differentiated from each other under the microscope.

Result : Anti-A ~~, Anti-B~~ and Anti-D showed agglutination.
My blood group is A+ve.

Questions

1. Different types of blood group systems.
2. Erythroblastosis fetalis
3. List out the blood transfusion reactions.

① The various blood grouping system includes ABO system, H-antigen, Rhesus system, MNS antigen system.
Out of which, in ABO system, there are 4 main blood groups A, B, AB and O. Each group can be either Rho positive or Rho negative which means in total there are 8 blood groups.

② Hemolytic disease of the fetus and new born also known as erythroblastosis fetalis. It is caused by the destruction of neonatal red blood cell by maternal Ig as antibodies. The formation of maternal antibodies in response to fetal antigen is called isoimmunization.

③ Types of transfusion reaction includes the following acute hemolytic, delayed hemolytic, febrile non hemolytic, anaphylatic, simple allergic, septic transfusion-related acute lung injury and transfusion-associated circulatory overload.

Experiment 15

Date :

RETICULOCYTE COUNT

AIM

To determine the blood reticulocyte count of the subject.

APPARATUS

Brilliant cresyl blue solution or new methylene blue, microscope, needle, cotton, sprit.

Staining solution : Composition

Brilliant cresyl blue - 1 gm

Sodium citrate - 0.4 gm

Sodium Chloride - 0.85 gm

Distilled water upto 100 ml

PROCEDURE

Wet Method : Mix one drop of blood and one drop of brilliant cresyl blue or new methylene blue on a glass slide. Put a cover glass. Wait for 10-20 minutes. Observe under oil immersion objective of the microscope. While counting, in order to prevent errors, the field is reduced. For this the eyepiece is removed and a piece of paper with a small central perforation is used in the eyepiece. Now only a few cells can be seen. Count 1000 RBCs and the number of reticulocytes per 1000 RBCs noted.

$$\frac{\text{No. of reticulocytes} \times 100}{\text{No. of RBC}}$$

gives the percentage of reticulocytes.

Reticulocytes

Reticulocytes are immediate precursors of red cell, they are called juvenile red cell.

(1) They are mature cells with full complement of hemoglobin

(2) They are slightly larger than RBC.

(3) They have a network of reticular nuclear material.

(4) The reticulum is stained by supravital stain

(5) Hemoglobin synthesis continues to some extent in same reticulocytes. The reticulocyte count is 0-1% of red cells in adults.

Reticulocytosis

physiological

(1) New born and infants

(2) High altitude

pathological

- Hemolytic anemia.
- Acute hemorrhage.
- During treatment of Fe deficiency anemia.
- Any conditions that stimulates the bone marrow to produce red cells.

Reticulocytopenia

* Aplastic anemia
* Myxoedema
* Hypopituitariasis

Reticulocyte response indicates a favorable response to a treatment. This is typically observed in treatment of pernicious anemia of Fe deficiency anemia. Reticulocyte provide information on anemia type.

Experiment 16 Date :

PLATELET COUNT

PRINCIPLE

In the direct method, a known volume of blood is diluted 200 times with diluting fluid which stains the platelets. The number of platelets present in a known volume of the diluted blood is counted and from it the number of platelets present in one cmm of blood is calculated.

APPARATUS

Hemocytometer with RBC pipette, Neubauer's double counting chamber, thick coverslip and diluting fluid.

Diluting fluid : Rees - Ecker fluid

Sodium citrate	-	3.8 gm	-	prevents coagulation, makes isotonic
Formalin	-	0.2 ml	-	fixative, prevents fungal growth
Brilliant cresyl blue	-	0.05 mg	-	identifies diluent

PROCEDURE

The left ring finger is pricked as usual and the blood is sucked exactly upto 0.5 mark in the RBC pipette. Immediately after this, the diluting fluid (Rees Ecker fluid) is sucked up to 101 mark. The blood is thoroughly mixed with the fluid by gently rotating the pipette. The first few drops are discarded and the counting chamber of the hemocytometer is charged with the diluted blood in the usual manner. The charged counting chamber is covered with a petridish containing a wet blotting paper. The chamber is allowed to remain for about 10 minutes so that the platelets may settle. The wet blotting paper prevents the drying up of the diluted blood present in the counting chamber. The platelets present in 25 small squares of the RBC counting area are enumerated under the high power of the microscope. The light should be partially cut down so that the platelets are visible. The number of platelets present in 1 cmm of undiluted blood is calculated as given below.

Calculation:

Suppose N be the number of platelets counted in 25 small squares of RBC counting area.

The area of 25 small squares is 1 sq mm

The depth between the counting area and converslip is 1/10 cmm.

So volume of fluid present in 25 small squares is 1/10 cmm.

As 1/10 cmm of diluted blood contains N number of platelets, 1 cmm of ciluted blood will contain 10 N number of platelets. The dilution of blood is 200 times.

Therefore 1 cmm of undiluted blood will contain 10N x 200 ie, 2000N number of platelets.

Precautions :

1. Platelets are carefully distinguished from foreign particles.
2. Diluting fluid should be filtered every time before use.

Normal count of platelet is 1.5-4 lakhs/mm³ of blood.

Platelet partiapate in the coagulation of blood and are therefore associated with hemostatic mechanisms of the Body.

Platelet count is usually ordered as a part of the laboratory diagnosis of a bleeding disorder. When platelet count decreases, it is thrombocytopenia. Thrombocytopenia can be caused by impaired production or increased destruction of ~~platelt~~ platelet. Prolonged bleeding is a hall mark of thrombocytopenia

Conditions that alter the thrombocyte count

Thrombocytosis

(1) Polycythemia vera.
(2) chronic meyeloid leukemia,
(3) Fe deficiency anemia, etc.

Thrombocytopenia

(A) Increased platelet destruction.
eg:- SLE

(B) Decreased production
eg:- Aplastic anemia, etc.